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OBJECTIVE: To evaluate the Intravenous Membrane Oxygenator or Hattler Catheter (IMO) in an ovine model of lung injury due to inhalation of chlorine gas. HYPOTHESIS: IMO will improve the PaO2-to-FiO2 (PFR) ratio in injured sheep. METHODS: Thirteen ewes (sham + IMO, n=1; injury + IMO, n=7; injury without IMO, n=5,) were used. Anesthetized sheep were ventilated with 300 L of 100 ppm chlorine (mixed in 100% O2). When animals reached ARDS (PFR<200), IMO was inserted in the injury + IMO group. ICU care, deep sedation, and mechanical ventilation were continued for up to 96 h. RESULTS: IMO was safely inserted in all cases. Gas exchange of the IMO was consistent at rates (normalized to a PCO2 of 50 mmHg) of 300-350 ml/min/m2. The IMO exerted a beneficial effect on PFR up to hour 18 and on PaCO2 up to hour 30. IMO use was associated with hemolysis, manifested by increased levels of plasma free hemoglobin. In order to reduce the anticipated impact of the HC on cardiac preload an aggressive approach to fluid management was employed, which likely worsened pulmonary edema and oxygenation. Thus, future studies employing the IMO will involve a fluid-sparing approach to management.

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# INTRODUCTION

This project represents a continuing collaboration between the U.S. Army Institute of Surgical Research (ISR), Fort Sam Houston, TX, and the McGowan Institute of Regenerative Medicine at the University of Pittsburgh, Pittsburgh, PA on testing of the intravenous membrane oxygenator (IMO) catheter or Hattler Catheter (IMO) developed at the University of Pittsburgh. The goal is to improve our ability to treat casualties with acute respiratory distress syndrome (ARDS). The objectives of this project are to evaluate the IMO catheter in an ovine model of severe ARDS secondary to inhalation of chlorine.

The reader is referred to previous reports on the development and characterization of the chlorine ARDS model. The work done during the past year was done on a no-cost extension to the current two-year protocol, and represents additional work done after completion of the main objectives of the original statement of work.

# BODY

## Part 1. Work Performed at the U.S. Army Institute of Surgical Research

### MIGET Study

Before testing the IMO in injured sheep we performed experiments to describe the pathophysiology hypoxia following chlorine inhalation injury by means of the Multiple Inert Gas Elimination Technique (MIGET).

### IMO Study

The IMO was then tested in injured sheep. This phase of the study began with training on the insertion of the Hattler Catheter that involved 4 acute (6-8 hour) studies. During these experiments the University of Pittsburgh and Alung teams trained ISR staff on insertion of the IMO and operation of the device console.

The combined effects of systemic heparinization and fluid loading at insertion of the IMO appeared to worsen the chlorine inhalation injury and to decrease survival times. We therefore used a relatively low dose of chlorine, 100 ppm mixed in 100% $O_2$. The 100 ppm dose led to development of ARDS within 2-3 hours after inhalation injury.

A total of 20 experiments were conducted. 4 animals were used for refinement of the chlorine dose and training in IMO insertion; 3 animals were excluded from the study as outliers (illness at the beginning of the experiment); 13 animals were included in the protocol. A detailed description of the work completed to date follows below.

### Regulatory Compliance

These studies were approved by the institutional Animal Care and Use Committee. The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other federal statutes and regulations relating to animals and studies involving animals, and by the 1996 *Guide for the Care and Use of Laboratory Animals* of the National Research Council. All animals were maintained in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care International.

### Personnel and Training

Personnel involved in this study had accumulated considerable experience in rendering round-the-clock animal intensive care as reflected in previous reports. No personnel changes are to be reported for this review period.

# MATERIALS AND METHODS

## MIGET Study Procedures

Experiments lasted 24 hours and involved doses of 0-90 ppm mixed with 21% $O_2$. The number of animals used and group-specific doses were: n=5, 0 ppm; n=5, 60 ppm; n=7, 90 ppm.

### Animal preparation

17 certified non-pregnant female sheep weighing 44.91 kg±1.1 SEM were quarantined for one week. On the day of study, the animals were anesthetized with isoflurane and underwent placement of a urinary catheter, tracheostomy and lines in the right external jugular vein (REJV), right carotid artery, and left and right femoral arteries and veins. Enrofloxacin (Bayer, Shawnee Mission, Kansas, USA), 100 g/ml, 1 ml BID IM, was given as prophylaxis on the day of surgery and every 24 h. At completion of surgery isoflurane was tapered off, and total intravenous anesthesia (TIVA) was initiated (ketamine, 300-500 mcg/kg/min; midazolam, 1 mcg/kg/min) and continued throughout the experiment. Anesthesia levels were adjusted based on pinch tests and clinical assessment. When indicated, additional buprenorphine (Buprenex) 0.3 mg/kg IM was given for pain. The animals were transported to the ICU and mechanically ventilated (see Ventilator Management below).

### Physiologic Measurements

Arterial blood gases (ABGs) were measured with an i-STAT blood gas analyser (Abbot Laboratories, East Windsor, NJ). A pulmonary arterial (PA) catheter (7 F, Model 41239-04-05, Abbott, North Chicago, IL) was inserted via the REJV. Bolus thermodilution cardiac output (CO) and pulmonary artery wedge pressure (PAWP) were determined at each time point. Electrocardiogram (ECG), pulse oximetry ($SpO_2$) (Datex Ohmeda True Tech Plus 3900), central venous pressure (CVP), peripheral arterial pressure (PAP), and arterial blood pressure (ABP) waveforms were continuously displayed using a clinical monitor (Viridia CMS 2000, Boeblingen, Germany). Pressures were transduced (Transpac® IV, Abbott, North Chicago, IL) at heart level. Physiologic data were digitally recorded at specified time points as shown in the outline of the protocol (Table 1).

Table 1

MIGET study: Experimental Protocol

| Time | Time Point | Event |
|---|---|---|

| | | |
|---|---|---|
| -2.0 h | Surgical preparation | TIVA, surgical line placement |
| 00.00 | Baseline | $FiO_2$ 21%, MIGET, physiologic data recording, ABG, CT |
| 0.5 h | Inhalation injury | 0, 60, 90 ppm $Cl_2$ injury in the negative pressure suite. Return to ICU |
| 0.5 h | 30 min post-injury | $FiO_2$ 21%, MIGET, physiologic data recording, ABG |
| 1.0 h | 1 h post-injury | $FiO_2$ 21%, MIGET, physiologic data recording, ABG |
| 2.0 h | 2 h post-injury | $FiO_2$ 21%, MIGET, physiologic data recording, ABG, CT |
| q6 h | Every 6 h post-injury | Vital sign collection, ABG |
| 24 h | 24 h post-injury | $FiO_2$ 21%, MIGET, physiologic data recording, ABG, CT, termination of experiment, necropsy |

*TIVA = induction of total intravenous anesthesia. $FiO_2$ = fraction of inspired oxygen, %. ABG = arterial blood gases. CT = computed tomography. MIGET = multiple inert gas elimination technique.*

The MIGET was carried out according to the method of Wagner et al. (15). Details of our MIGET technique have been described elsewhere (2). Briefly, a 1 L bag of 5% dextrose was saturated by six inert gases: $SF_6$, ethane, cyclopropane, halothane, ethyl ether and acetone. This infusate was administered IV at a constant rate of half the minute ventilation rate expressed in ml/min. During sampling time points, 7 ml of arterial blood and 30 ml of mixed expired air were collected into air-tight glass syringes. Gas chromatography (GC) was used to determine the levels of the inert gases in the expired air and arterial blood. The obtained GC, ABG, oxygen consumption ($VO_2$), carbon dioxide production ($VCO_2$), CO, minute ventilation (Ve), body temperature, room temperature and individual inert gas solubility data (determined experimentally for each of the gases) were entered into custom software provided by Dr. Wagner. Mixed venous levels of the six gases were calculated by the software. The retention (ratio of the arterial to mixed venous levels) and excretion (ratio of the expired to mixed venous levels) of each gas were represented as a function of solubility in blood. Ventilation-perfusion ratio (V/Q) was assessed graphically and numerically.

General ICU care was identical as reported below for the IMO study procedures with the following exceptions:

1. Fluid management: urine output was maintained according to the 4-2-1 rule as specified in the IMO procedures section. No other fluids were administered.
2. Ventilator management: A Servo 300-A (Siemens-Elemia, Sweden) mechanical ventilator was used. Volume control mode was used with 5 cm $H_2O$ positive end expiratory pressure (PEEP) and a goal for oxygenation of at least 90% by arterial

blood hemoglobin $O_2$ saturation ($SpO_2$ and/or $SaO_2$). The fraction of inspired $O_2$ concentration was 21% and was adjusted as necessary to maintain $SpO_2 \geq 90\%$ and partial pressure of $O_2$ in arterial blood ($PaO_2$) > 60 mm Hg. At least two hours before MIGET sampling, the $FiO_2$ was decreased to 21%. PEEP was not modified.

The target for peak inspiratory pressure (PIP) was ≤ 40 cm $H_2O$. The target for pH was ≥ 7.25. Baseline TV settings were 13 ml/kg. If arterial pH was greater than 7.25, TV was lowered by 2-3 ml/kg steps to keep PIP below 40 cm $H_2O$. If pH was below 7.25, decreases in TV were avoided, and a higher PIP was accepted. Baseline RR was 15/min. TV and RR were adjusted to maintain the arterial level of $CO_2$ ($P_aCO_2$) between 30 and 45 mm Hg. To prevent atelectasis, when in hypocapnia and low to normal PIP, the RR was dialed down before TV adjustments were made. If PIP was high, TV was decreased first to keep PIP below 40 cm $H_2O$ followed by RR adjustments.

## IMO Study Procedures

Thirteen certified non-pregnant female sheep were used. They were quarantined for one week. There were 8 in the injured + IMO group, weighing 59.75 kg ± 1.21 SEM. There were 5 in the injured, non-treated group weighing 49.16 kg± 0.7 SEM.

On the day of study, the animals were anesthetized with isoflurane and underwent placement of a urinary catheter, tracheostomy and lines in the right external jugular vein, right carotid artery, and left and right femoral arteries and veins. At completion of surgery, isoflurane was tapered off, and total intravenous anesthesia (TIVA) was initiated (ketamine, 300-500 mcg/kg/min; midazolam, 1 mcg/kg/min) and continued throughout the experiment. Anesthesia levels were adjusted based on pinch tests and clinical assessment. When indicated, additional buprenorphine (Buprenex) 0.3 mg/kg IM was given for pain. The animals were transported to the ICU and mechanically ventilated (see Ventilator Management below). Enrofloxacin (Baytril, Bayer, Shawnee Mission, Kansas), 100 g/ml, 1 ml BID IM, was given as prophylaxis against pneumonia on the day of surgery and every 24 h. This was done because of the results of early histopathological and microbiological analyses indicating a high risk of pneumonia secondary to *Pseudomonas aeruginosa*.

### Physiologic Measurements

Arterial blood gases were measured at baseline, immediately after injury, and every 6 hours. A point-of-care analyzer was used (i-STAT portable clinical analyzer, #06F16-02; CG8+ cartridges, #220400; Abbot Laboratories, East Windsor, NJ). Temperature-corrected data are reported.

A pulmonary arterial (PA) catheter (Thermodilution Catheter, Torque-line, 7 F, 4-lumen, #41239-04-05, Abbott Laboratories, North Chicago, IL) was inserted via the right external jugular introducer. Arterial and venous catheters were kept patent with heparin,

40 units/ml, 2-6 ml/flush. Pressures were transduced (Transpac IV trifurcated monitoring kit, #42650-06, Abbott Critical Care Systems, North Chicago, IL) with the transducers level with the sheep's phlebostatic axis. The electrocardiogram, the pulse oximetry plethysmogram, and the central venous, pulmonary arterial, and systemic arterial blood pressure waveforms were continuously displayed using a Hewlett Packard clinical monitor (Model 88 M1176A).

At baseline, immediately after injury, and every 6 hours, the above waveforms were acquired at 500Hz to the DREW data acquisition system developed at ISR (Millar Instruments, Houston, TX) (12). The cardiac output was determined by thermodilution, and the pulmonary capillary wedge pressure was measured.

$Cl_2$ Injury

Inhalation injury occurred in a dedicated suite under negative-pressure conditions (4). Ambient sampling was used (detector head GM-PS-6A-H; sensor GM-CDS-6-CL10-R; Matheson Tri Gas, Chicago, IL) to detect gas leaks (none occurred). Personnel performing $Cl_2$ delivery wore full-face fitted gas masks. A custom gas mix consisting of $Cl_2$ 1000 ppm, balance medical air, was obtained (#G2659698, Matheson Tri Gas). Using a mass flow blender (MMIX-0116-XX, Matheson Tri Gas) the gas was diluted to yield 100 ppm $Cl_2$ concentration with 100% medical $O_2$. $Cl_2$ was delivered via tracheostomy with an Ambu bag at a tidal volume (TV) of 800 ml, respiratory rate (RR) of 10/min for 30 min, plus 200 ml free flow through the circuit, yielding 300 liters. Expired air was passed through a Boeringer scavenger to a charcoal canister (Precision Filtration Products, Pennsburg, PA), and was evacuated via the institutional vacuum system. After exposure to $Cl_2$, the animals were transported back into the OR and monitored.

ICU Management Post-injury

TIVA was carried out during the study rendering the subjects unconscious throughout the duration of the protocol. A similar standard of care was applied to both groups of animals. When the MAP decreased below 50 mm Hg despite resuscitation, dopamine was initiated at a rate of 2 mcg/kg/min. Over the duration of the study the rate was adjusted up to 20mkg/kg/min. Because of the large fluid load post injury and to prevent the development of jatrogenic pulmonary edema diuretics (Furosemide and Mannitol in one case)) were used (5-10 mg bolus every 2 hours) to stimulate urine output. Animals that developed decreased HR and/or bradycardia (HR< 60 beats/min) received antiarrhythmic interventions that included boluses of atropine, lidocaine and isoproterenol at standard recommended doses that were regulated by the attending veterinarian.

Fluid Management

At initiation of the experiment each animal was placed on a 5 ml/kg continuous rate infusion of lactated Ringer's solution (LR). As expected based on our model

development data the animals developed hypotension after chlorine inhalation which was treated with further LR infusion at 5-10 ml/kg hour. Additionally, placement of the IMO required a fluid load of 10-15 ml/kg. For these two reasons, the animals received a standard of 10-15 ml/kg LR after injury and during IMO placement. After that, intravenous fluids were given according to the following program. The maintenance i.v. fluid rate was calculated using the 4-2-1 rule: 4 ml/kg for the first 10 kg of body weight, 2 ml/kg for the next 10 kg, and 1 ml/kg for each additional kg. The maintenance i.v. fluids included 5% dextrose in water (D5W) at one-half the maintenance rate, plus normal saline (NS) at one-half the maintenance rate. Additional NS was given to maintain a urine output of 0.5 to 1 ml/kg/h. If the hourly urine output was less than 0.5 ml/kg/h, the NS was increased by 25%. If the hourly urine output was more than 1 ml/kg/h, the NS was decreased by 25%. The NS was not decreased below the maintenance rate. The D5W was adjusted only to treat hyponatremia or hypernatremia, which were rare. For these infusions, a dual channel volumetric infusion pump (Alaris Signature Gold IVAC, #7230) was used.

Ventilator Management

A Servo 300-A (Siemens-Elemia, Sweden) mechanical ventilator was used. Volume control mode was used with an initial PEEP of 0 cm $H_2O$. The goal for oxygenation was $SpO_2$ and/or $SaO_2 \geq 90\%$ and $PaO_2 > 60$ mm Hg. At baseline, the $FiO_2$ was set at 21%, and it was adjusted as needed to maintain these goals. In general, an effort was made to set the PEEP above the lower inflection point on the pressure-volume curve. In addition, the method published in the ARDSNet trial was used to select the maximum allowable PEEP based on the $FiO_2$ (table 1, ref 1).

A more aggressive approach to lung-protective ventilation was employed than in the MIGET study. The target for peak inspiratory pressure (PIP) was $\leq 30$ cm $H_2O$. The target for pH was $\geq 7.25$. Baseline TV settings were 13 ml/kg. After injury, low tidal volume ventilation and permissive hypercapnia was used for all animals, as follows: if PIP $\geq 30$ cm $H_2O$, TV was lowered by 2-3 ml/kg steps to achieve PIP $< 30$ cm $H_2O$. But, if $< 7.25$, decreases in TV were avoided, and a higher PIP was accepted.

IMO Insertion

When the $PaO_2$-to-$FiO_2$ (PFR) reached 200 as an indicator of development of ARDS, the procedure for the IMO (8-10, 13) placement was initiated. First, a 25 u/kg heparin bolus was carried out. The ACT target prior to insertion was 220-250 sec. Next, the IMO was inserted into the left external jugular vein via a 10 cm skin cutdown and advanced to the inferior vena cava with the proximal portion of the fibers spanning the right ventricle. The correct position was verified by fluoroscopy. Catheter balloon pulsation was initiated immediately upon placement. Skin was closed with staples and an aseptic dressing.

The first 5 IMOs were placed with the animals in the dorsally recumbent position. This was associated with hypotension, which was attributed to the rumen pressing on the major veins and impeding venous return. Because of this, we developed and adopted a new technique of IMO placement that was carried out in the prone position. This change in insertion was hemodynamically advantageous and was adopted as a standard procedure.

Experiment Termination, Necropsy

Ninety-six hours after injury or sooner in the event of imminent death [MAP <30 mm Hg for 30 min], animals were euthanized by an overdose of sodium pentobarbital (Fatal-Plus, Dearborn, MI). Dorsal sections of the middle lobe in the right lung were harvested, fixed in formalin and processed for H & E staining and light microscopical examination.

## RESULTS

### MIGET Study

A total of 408 hours of ICU time were required to complete the MIGET study. All animals survived. $PaO_2$ was nearly halved at 30 min and continued to drop reaching a nadir at 2 hours (Table 2). As we noted previously for higher doses, there was an improvement in $PaO_2$ around 12-24 h, returning to levels not significantly different than baseline by 24 hours in group 2 (60 ppm) (Table 2).

Table 2

MIGET Study: Ventilation-Perfusion Data

| Variable | Group | 0 h | 30 min | 1 h | 2 h | 24 h |
|---|---|---|---|---|---|---|
| **$PaO_2$** | **1** | 97.3±4.0 | 100.1 ±6.7 | 99.9 ±8.2 | 102.0 ±4.9 | 87.1 ±2.0 |
| | **2** | 92.1 ±2.3 | 55.0 ±5.6 | 49.8 ±6.2[b] | 47.3 ±3.3 | 70.2 ±15.0 |
| | **3** | 92.3 ±6.7 | 48.6 ±5.2 | 40.9 ±2.7[c] | 44.4 ±3.7 | 59.0 ±6.2[a] |
| **$\dot{Q}$ true shunt** | **1** | 1.02 ±0.27 | 1.58 ±0.55 | 2.13 ±0.86 | 2.08 ±0.90 | 2.38 ±0.88 |
| | **2** | 2.02 ±0.67 | 14.18 ±4.37 | 33.22 ±4.39[b] | 45.22 ±4.93 | 39.84±6.07[b] |
| | **3** | 3.31 ±0.73 | 23.13 ±8.78 | 37.17 ±7.07[b] | 45.24 ±6.48 | 44.50 ±6.66[b] |
| **$\dot{Q}$ V/Q 0 to 0.01** | **1** | 0.00 ±0 | 0.00 ±0 | 0.00 ±0 | 0.00 ±0 | 0.00 ±0 |
| | **2** | 0.00 ±0 | 10.72 ±1.94 | 8.28 ±2.22[a] | 1.26 ±0.83 | 0.32 ±0.32 |
| | **3** | 0.00 ±0 | 9.93 ±1.18 | 9.83 ±1.22[c] | 4.66 ±0.95 | 0.79 ±0.48 |
| **$\dot{Q}$ V/Q 0.01 to 0.1** | **1** | 0.00 ±0 | 0.00 ±0 | 0.00 ±0 | 0.00 ±0 | 0.00 ±0 |
| | **2** | 4.22 ±2.01 | 13.64 ±3.93 | 8.82 ±2.76 | 0.94 ±0.65 | 1.50 ±1.03 |
| | **3** | 1.47 ±0.85 | 16.16 ±1.14 | 12.96 ±2.68[a] | 5.76 ±1.74 | 1.61 ±0.96 |
| **$\dot{Q}$ V/Q 0.1 to 1** | **1** | 90.12 ±3.14 | 92.75 ±4.21 | 93.13 ±4.20 | 92.62 ±2.26 | 84.68 ±7.09 |
| | **2** | 87.60 ±3.29 | 49.78 ±9.50 | 39.50 ±8.51[a] | 39.98 ±7.92 | 35.06 ±8.07[a] |
| | **3** | 88.44 ±2.44 | 35.99 ±9.27 | 25.14 ±4.97[c] | 21.89 ±3.55 | 36.34 ±7.39[c] |
| **$\dot{Q}$ V/Q 1 to10** | **1** | 8.64 ±3.32 | 5.48 ±4.50 | 4.53 ±4.43 | 5.10 ±2.55 | 12.86 ±6.80 |
| | **2** | 6.12 ±1.97 | 11.00 ±2.52 | 9.48 ±2.15 | 11.62 ±2.57 | 22.74 ±5.37 |
| | **3** | 14.89 ±8.73 | 13.97 ±3.52 | 14.43 ±2.31 | 21.07 ±4.02 | 16.40 ±1.71 |
| **$\dot{Q}$ V/Q 10 to 100** | **1** | 0.16 ±0.08 | 0.18 ±0.18 | 0.20 ±0.07 | 0.16 ±0.07 | 0.10 ±0.10 |
| | **2** | 0.00 ±0 | 0.66 ±0.20 | 0.76 ±0.08 | 0.96 ±0.23 | 0.52 ±0.29 |
| | **3** | 0.36 ±0.15 | 0.84 ±0.15 | 0.47 ±0.17 | 1.41 ±0.37 | 0.30 ±0.17 |
| **Mean $\dot{Q}$** | **1** | 0.62 ±0.01 | 0.58 ±0.06 | 0.58 ±0.06 | 0.61 ±0.05 | 0.57 ±0.10 |
| | **2** | 0.39 ±0.03 | 0.24 ±0.07 | 0.26 ±0.07 | 0.61 ±0.05 | 0.87 ±0.15[a] |
| | **3** | 0.49 ±0.05 | 0.19 ±0.03 | 0.21 ±0.03[a] | 0.55 ±0.13 | 0.68 ±0.06 |
| **$logSD_{\dot{Q}}$** | **1** | 0.45 ±0.04 | 0.40 ±0.05 | 0.42 ±0.02 | 0.39 ±0.04 | 0.51 ±0.04 |
| | **2** | 0.70 ±0.10 | 1.84 ±0.17 | 1.81 ±0.27 | 1.13 ±0.25 | 0.89 ±0.23 |
| | **3** | 0.61 ±0.06 | 2.93 ±0.83 | 2.21 ±0.07[c] | 1.96 ±0.15 | 1.08 ±0.18 |
| **Skewness $\dot{Q}$** | **1** | 0.20 ±0.07 | 0.18 ±0.12 | 0.25 ±0.08 | 0.18 ±0.08 | 0.05 ±0.07 |
| | **2** | -0.09 ±0.07 | -2.64 ±1.67 | -2.70 ±1.44 | -2.14 ±1.93 | -1.27 ±1.50 |
| | **3** | 0.15 ±0.17 | -1.22 ±1.86 | -3.54 ±1.13[a] | -5.62 ±0.90 | -1.74 ±0.97 |

*Table 2. Group 1 is sham injury, group 2 is 60 ppm, group 3 is 90 ppm. Letters indicate p values for post-hoc paired-samples t tests, comparing data at hour 1 and hour 24 to hour 0: a, p<.05; b, p<.01; c, p<.001. $PaO_2$, partial pressure of oxygen in arterial blood, mm Hg. $Q_{true\ shunt}$, percentage of the cardiac output (Q) pertaining to the true shunt compartment, V/Q=0. $Q_{V/Q\ 0\ to\ 0.01}$, Q to the compartment for which the V/Q ratio is between 0 and 0.01, etc. Mean V/Q, mean value of V/Q on a logarithmic scale for the function, Q = f(V/Q) . $logSD_Q$, second moment (logarithmic standard deviation) of this function. Skewness Q, third moment of this function.*

The most striking finding from the MIGET analysis was an early increase in blood flow to the true shunt compartment in both injury groups, which was sustained through 24 h (Table 2). There was also an increase in blood flow to the very low (V/Q 0 to 0.01) and low (V/Q 0.01 to 0.1) compartments, which, by contrast with true shunt, resolved by 24 h (Table 2). This redistribution of blood flow to shunt, very low, and low V/Q compartments occurred at the expense of blood flow to the normal V/Q compartment (V/Q 0.1 to 1). Meanwhile, blood flow to the high (V/Q 1 to 10) and very high (V/Q 10 to 100) compartments did not change (Table 2). Marked dispersion and skewness of V/Q distributions were present after $Cl_2$ inhalation as evidenced by increased Log SDQ above 0.6 (normal level) and in many cases above 2.0, attesting to injury severity (Table 2). Figure 1 depicts examples of V/Q changes over time in an injured subject.

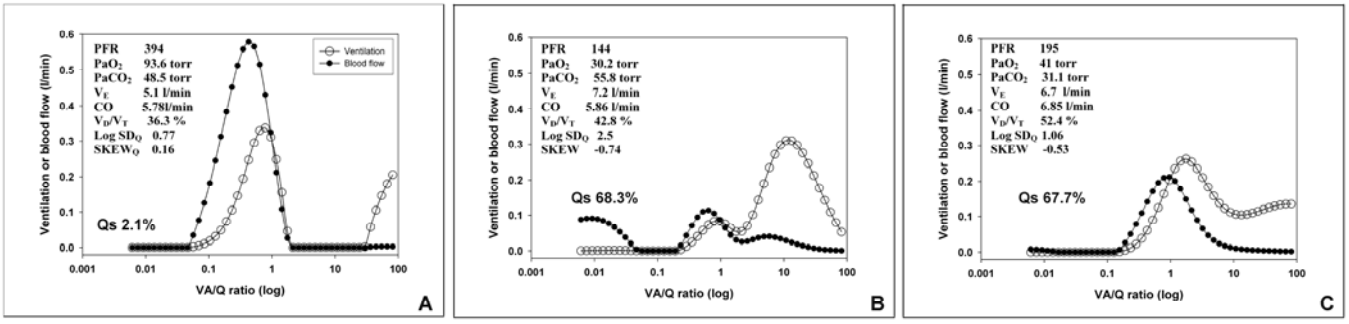


*Figure 1. Distributions of ventilation (V) and perfusion (Q) as a function of V/Q ratio in an animal exposed to 90 ppm of $Cl_2$. **A.** Baseline. V/Q ratio is normal and centered around 1. V and Q distributions are well matched and the amount of true shunt is low and within normal physiologic limits. **B.** 2 h post-injury. Note severe V/Q mismatch, blood flow to very low and low V/Q areas and a true shunt of 68% of CO, marked dispersion (scatter) and skewness of the V and Q distributions. **C.** 24 h post-injury. Shunt remained unchanged however the V/Q matching improved as the blood flow to low and very low V/Q areas became redistributed to normally aerated segments of the lung.*

Increases in dead space ventilation and diffusion limitation were not features of this injury. The excellent correlation ($r^2$=0.9959) between the measured arterial $PO_2$ and the arterial $PO_2$ predicted by the MIGET proves that full alveolar arterial equilibration took

place at times of sampling, thus excluding diffusion limitation as a cause of hypoxia in this model.

## IMO Study

IMO did not influence the main hemodynamic parameters upon insertion and over the course of the experiment when compared to the injury group as heart rate (HR), mean arterial pressure (MAP) and cardiac output (CO) stayed similar between groups (see Figures 5,6, 7)

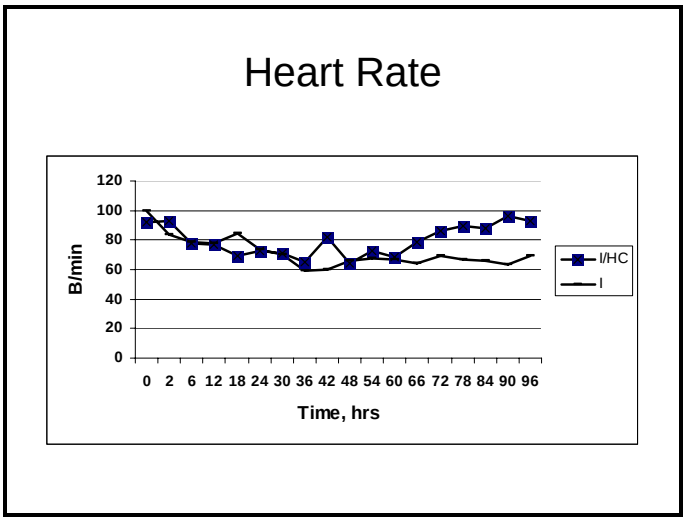


*Figure 2. Changes in heart rate. Squares: injury + IMO group. Line: injury group.*

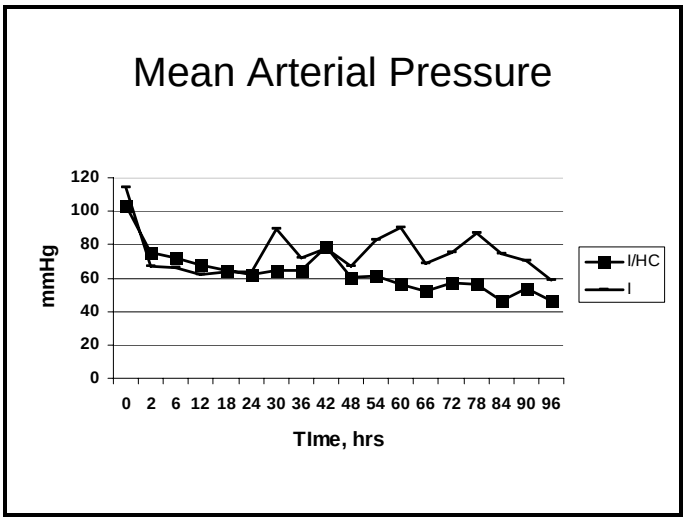


*Figure 3. Changes in heart rate.*

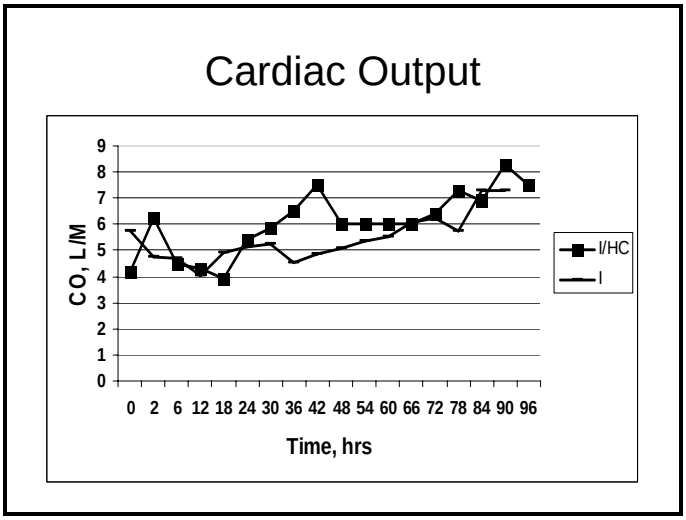


*Figure 4. Changes in cardiac output.*

IMO appeared to exert a beneficial effect on the PFR (up to hour 18) which was lost thereafter (see Figure 5). It is possible that the lung injury was worse in the IMO animals, e.g. secondary to the effects of hemolysis that was present in IMO-treated animals.

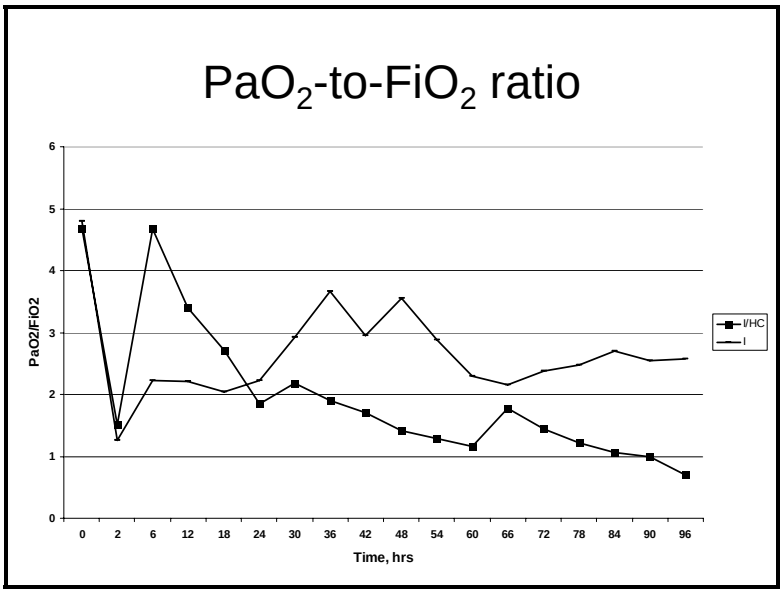


*Figure 5. Changes in $PaO_2$-to-$FiO_2$ ratio.*

Compared to untreated animals, the IMO exerted a marked effect on $PaCO_2$ during the first 36 h post injury (Figure 6).

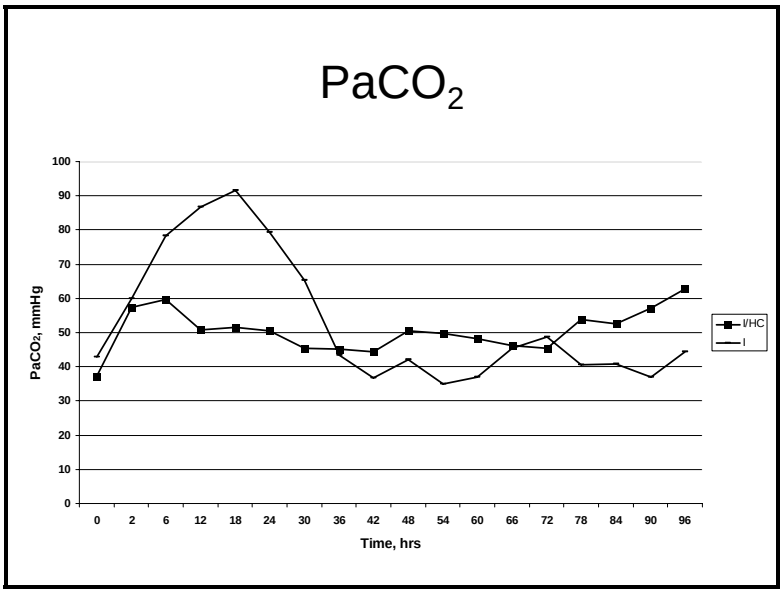


*Figure 6. Changes in $PaCO_2$.*

Fluid loading worsened the severity of injury, although the wedge pressure was in the 8-14 range (Figure 7).

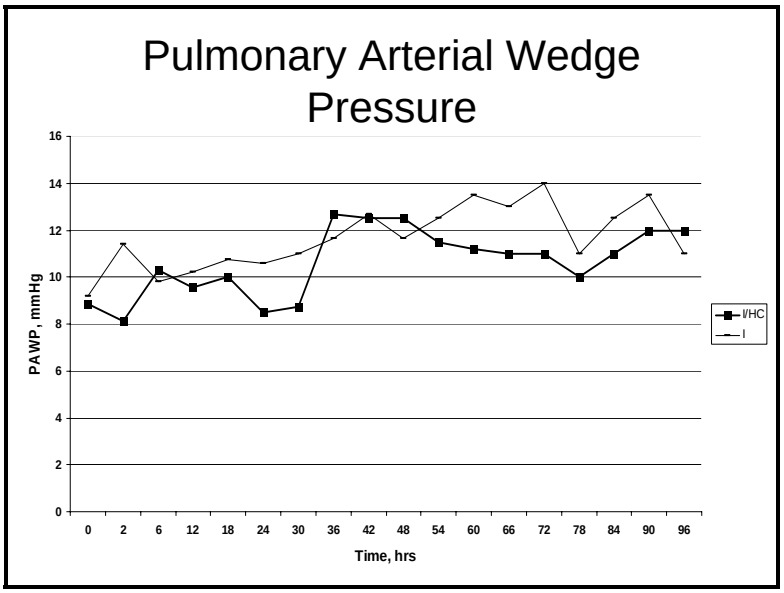


*Figure 7. Changes in pulmonary artery wedge pressure.*

IMO use was associated with metabolic acidosis manifesting in decrease in base excess. The etiology is unknown (see Figure 8).

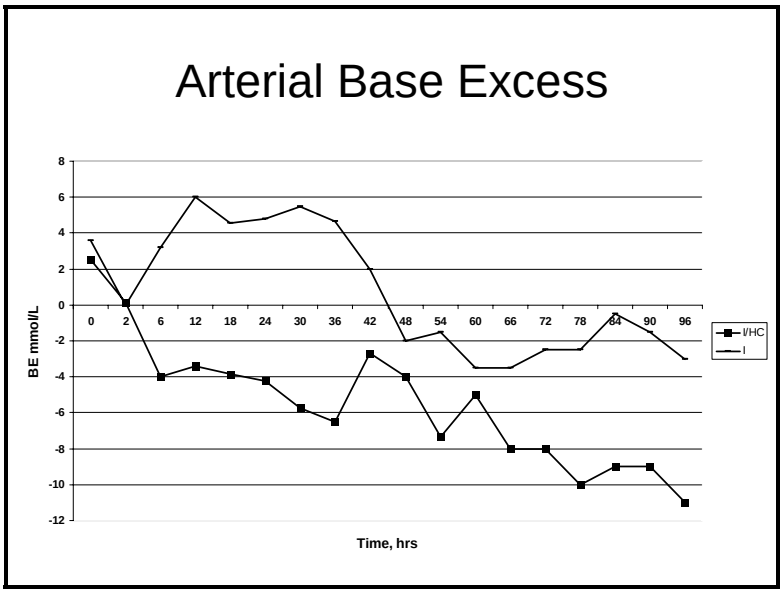


*Figure 8. Changes in arterial base excess.*

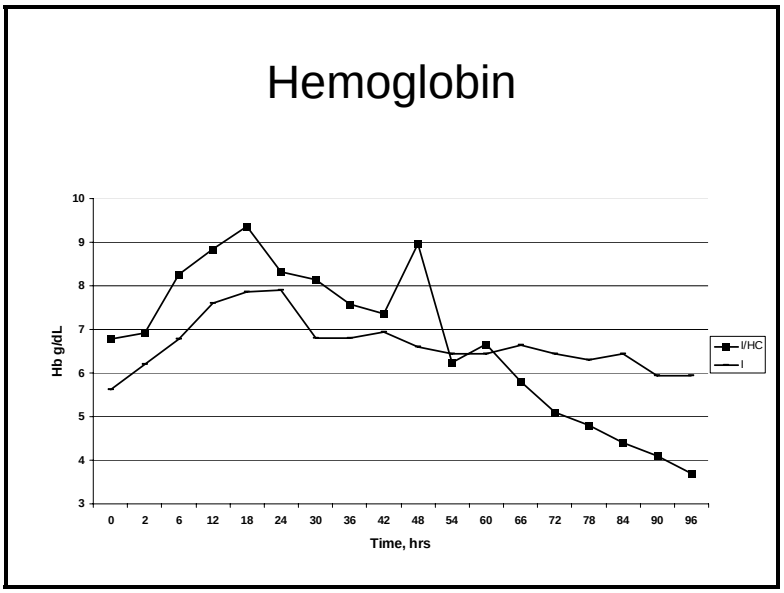


*Figure 9. Hemoglobin concentration.*

IMO Performance

Average gas exchange of the IMO was reasonably consistent among the n=8 animals receiving the IMO (n=7 injured, n=1 uninjured). Most of the animals had average exchange rates normalized to $PCO_2$ of 50 mmHg of 300-350 ml/min/$m^2$ (see Figure 13). Two animals were in the 250-300 ml/min/$m^2$ range. With the exception of one animal, there did not appear to be a consistent degradation in gas exchange over time as was seen in past healthy calf studies of the catheter performed at the University of Pittsburgh. When compared to previous studies in calves, the catheters explanted from the sheep in this study had much less thrombi accumulated on the bundle. Thrombus was typically limited to areas at or immediately subjacent to the support threads of the fiber fabric.

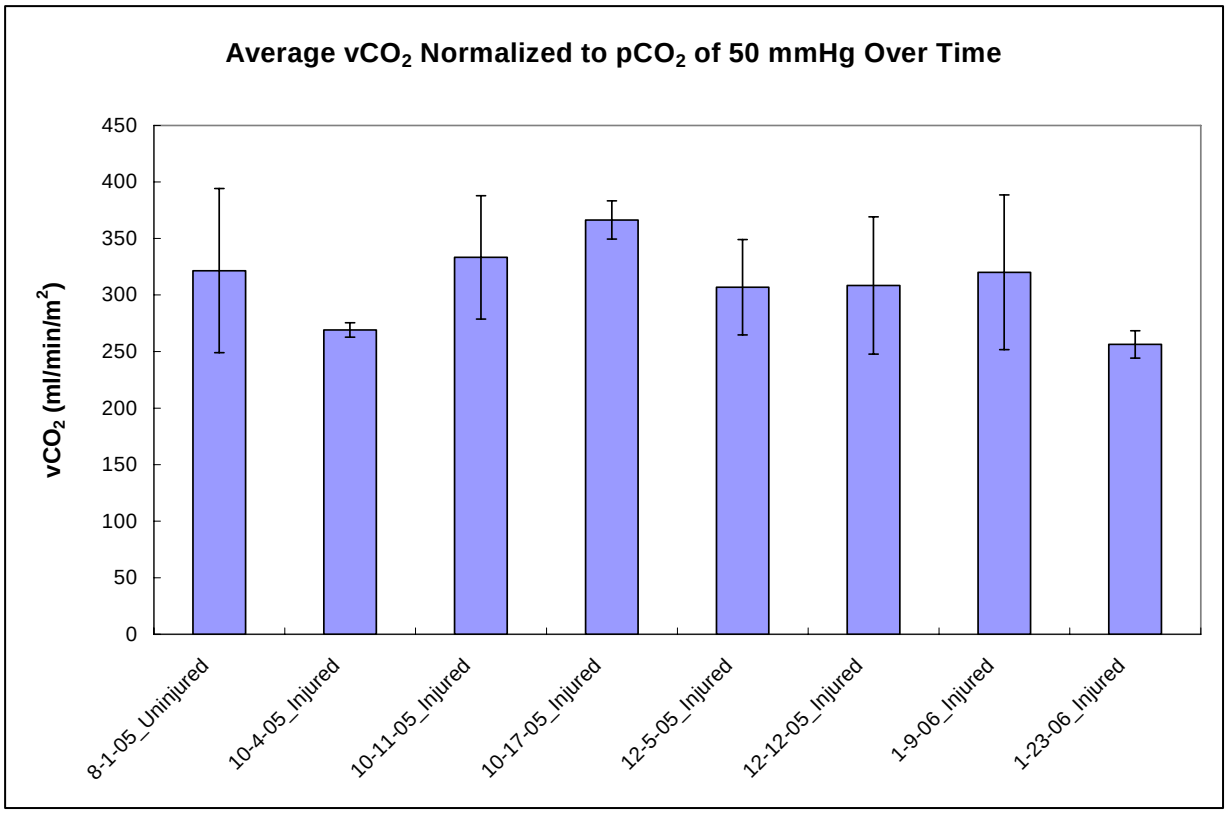


*Figure 10. Average normalized $CO_2$ elimination by the IMO.*

There were no significant failures of the catheter or the console during any of the sheep studies. No studies were terminated due to problems with the catheter or console. Plasma free hemoglobin in the animals receiving the IMO was generally high (see Figure 11). The 4 animals that went 48 hours had values of 150-350 mg/dL at 48 hours, and the two animals that went the 96 hours ended up with plasma free hemoglobin between 200 and 300 mg/dL.

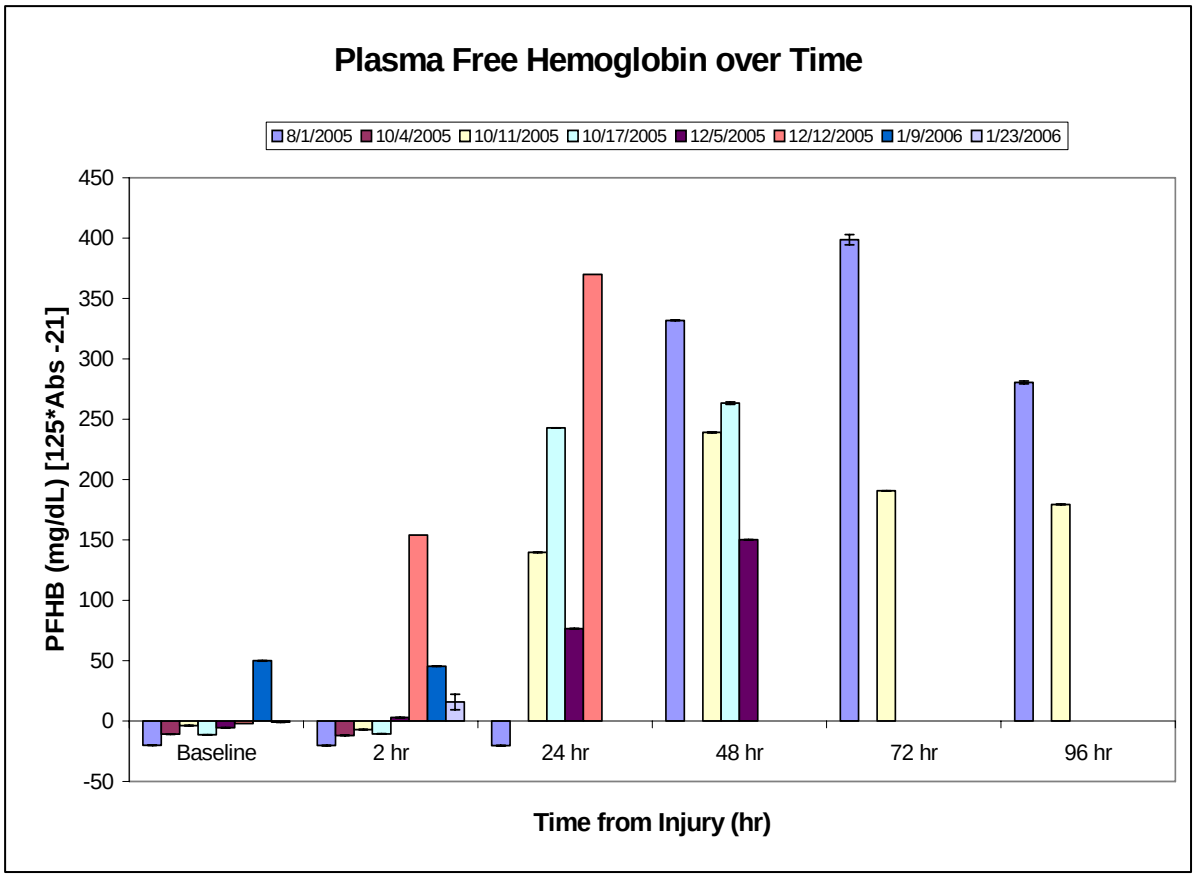


*Figure 11. Plasma free hemoglobin.*

# DISCUSSION

## MIGET Study

Pathophysiologically, $Cl_2$ causes an injury with features seen in both smoke inhalation (small airway lesions manifested by V/Q mismatch), and in ARDS secondary to systemic disease and pulmonary contusion (alveolar-endothelial lesions featuring an increase in true shunt).

## IMO Study

This phase of the study is limited by the small n surviving to the end of the protocol (96 h). Thus, the preliminary findings should be interpreted with caution. IMO appeared to exert a beneficial effect on $PaO_2$-to-$FiO_2$ ratio (PFR) (up to hour 18) which was lost thereafter. IMO exerted a beneficial effect on $PaCO_2$ during the first 30 hours. IMO use was associated with hemolysis, most likely due to fragility of sheep RBCs (11). Fluid loading may have worsened the severity of injury, although the wedge pressure was in the 8-14 range. IMO use was associated with metabolic acidosis (low BE). The etiology is unknown, although this may have represented renal tubular acidosis secondary to non-oliguric renal failure caused by pigmenturia. The median survival times were 37 +/- 11 (SEM) for the injured + IMO group and 49 +/- 1 (SEM) for the injury group. Mechanical ventilation parameters in the IMO and non-IMO animal groups were similar.

Methodologically, this study differed from the model development phase (Phase 1, reported earlier.) This phase involved creation of a dose-responsive model of chlorine inhalation injury (4). The fluid management in Phase 1 differed significantly from the approach in the current study involving the IMO. Namely, all animals in Phase 1 received a continuous infusion to maintain a urine output level of 0.5-1 ml/kg/hr and fluid boluses were not used. It is apparent that the 100 ppm dose of chlorine used in the current study appeared to exert a much more severe injury in the first 6 hours of the experiment, an injury consistent with a much higher dose. We propose that this discrepancy in injury severity was caused by the fluid load before, during and immediately after chlorine inhalation injury and during the IMO insertion. Most importantly, over the course of this study we established that the IMO can be inserted in prone position and, most likely, the MAP can be supported by dopamine as an alternative to fluid loading if necessary.

## Part 2. Work Performed by the University of Pittsburgh and by ALung Technologies, Inc.

Our collaborators from the University of Pittsburgh and ALung Inc. conducted on-site training in IMO insertion at the ISR during which the ISR team learned:

1. IMO insertion in supine position.

2. Use of fluoroscopy for verification of the IMO position in the heart and major vessels.

3. How to operate and troubleshoot the IMO console.

4. To conduct beat rate protocols and evaluate performance by the IMO.

We had continuous consultations on the progress of the study with our collaborators.

## KEY RESEARCH ACCOMPLISHMENTS

- The IMO was successfully and safely inserted in all cases.

- A safe, effective and hemodynamically advantageous method for insertion of the IMO in the prone position was developed and successfully carried out in all cases.

- Protocols for the critical care management of severely injured sheep were further developed and refined, including sedation, mechanical ventilation and fluid resuscitation.

- Minimally acceptable (ACT of 200-220 sec) systemic heparinization rates were developed. Levels considered to be full heparinization are ACT 350-450. Further studies will involve further reductions in the level of systemic heparinization with the aim to avoid systemic heparinization altogether.

- Gas exchange performed by the IMO was acceptable.

- Worsening of the pulmonary damage and hypoxia due to vigorous fluid resuscitation was documented. These findings reiterate the importance of judicious fluid management in patients with pulmonary failure. Alternatively, hemolysis may have contributed to the lung injury.

## REPORTABLE OUTCOMES

Peer-Reviewed Publications:

Batchinsky AI, Martini DK, Jordan BS, Dick EJ, Fudge J, Baird CA, Hardin DE, Cancio LC. Acute respiratory distress syndrome secondary to inhalation of chlorine gas in sheep. J Trauma. 2006;60:944-957.

Peer-Reviewed Presentations:

Dubick MA, Cameron DG, Batchinsky AI, Cancio LC. Indices of antioxidant status in sheep after exposure to chlorine gas. Society of Toxicology Annual Meeting, 5-9 Mar 2006, San Diego, CA.

Cancio LC, Batchinsky AI, Martini DK, Jordan BS, Dick EJ, Fudge J, Baird CA, Lucas M, Hardin DE. Acute respiratory distress syndrome secondary to inhalation of chlorine gas. American Association for the Surgery of Trauma Annual Meeting, 22-24 Sep 2005, Atlanta, GA.

Other Presentations:

Batchinsky AI, Federspiel B, Hattler BG, Martini DK, Jordan BS, Baird CA, Hardin BD, Roukous C, Eash H, Holcomb J, Cancio LC. Evaluation of the Hattler catheter in the treatment of ARDS secondary to chlorine inhalation. Presented at the U.S. Army Advanced Trauma Applications for Combat Casualty Care meeting in St. Petersburg Beach, FL, 2005.

## CONCLUSIONS

In sheep, exposure to $Cl_2$ resulted in: 1) pathophysiologically, the development of true shunt and V/Q mismatch; 2) clinically, a dose-dependent decrease in PFR, transient hypotension and decreased cardiac output; and 3) morphologically, a typical "feathery" appearance on CT with progressive development to confluent consolidation in the lung, reflecting extensive alveolar flooding and necrosis. (CT scan findings were described in earlier Reports.)

The IMO exerted a beneficial effect on $PaO_2$-to-$FiO_2$ ratio up to hour 18 and on $PaCO_2$ up to hour 30. IMO use was associated with hemolysis, most likely due to the intrinsic fragility of sheep red blood cells; plasma free hemoglobin in the animals receiving the IMO was generally high. In addition, an aggressive approach to fluid management was employed in these animals, in order to reduce the possible impact of the IMO on cardiac preload. However, it became evident that volume loading, in the presence of chlorine-induced alveolar-capillary membrane injury, worsened pulmonary edema and oxygenation.

Based this experience, future studies will include the following objectives:

- Our next series of animals employing the IMO will employ a fluid-sparing approach to management and the use of dopamine as required for blood pressure support.

- The IMO is currently in clinical trials for respiratory failure in the UK.

- The IMO will be completely redesigned as the Percutaneous Respiratory Assist Cathether (PRAC). This device is currently under development at the University of Pittsburgh. It will employ a completely new propulsion system, featuring rotation rather than balloon pulsation. This should greatly increase gas exchange efficiency and enable a substantial size reduction.

- An external device, the Paracorporeal Respiratory Assist Lung (PRAL) has also been developed at the University of Pittsburgh. This device is similar to arteriovenous CO2 removal devices such as the Interventional Lung Assist (ILA, Novalung), but involves a single percutaneous dual lumen catheter placed into the right atrium. Venovenous gas exchange is enabled by a rotating fiber bundle, which both propels blood and enhances gas exchange. This device will be tested at the U.S. Army Institute of Surgical Research in injured animals when new funds become available.

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